

MORPHOLOGICAL DIFFERENTIATION AMONG MADREAN SKY ISLAND POPULATIONS OF *CASTILLEJA AUSTROMONTANA* (SCROPHULARIACEAE)

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ABSTRACT

Populations of *Castilleja austromontana* (Scrophulariaceae) occur at high elevations (>2050 m) on the Madrean sky islands of southern Arizona, adjacent New Mexico and northern México. These mountain-top habitat islands are cooler and wetter than the desert and semi-desert grasslands below. We examined 41 vegetative, floral and seed characters for plants from six sky islands in Arizona. Univariate and multivariate analyses indicate strong morphological differentiation among populations. Discriminant functions analysis placed 88% of plants correctly, indicating that plants from different populations are morphologically distinct. Selection may contribute to the observed differentiation, but we have no basis for inferring the direction or strength of selection on vegetative traits. Sky island populations apparently share the same pollinators, so divergent selection on floral traits seems unlikely. Past and present gene flow may also be involved as our results conform, at least in part, to an isolation by distance model. The magnitude of character differences combined with inferences of population sizes suggests that genetic drift may also contribute to the differentiation observed.

Evolutionary biologists have long recognized that islands, both real and habitat, provide special opportunities to study population differentiation and speciation due to geographic isolation (MacArthur and Wilson 1967; Carlquist 1974; Grant 1986, 1998 and papers therein; Wagner and Funk 1995 and papers therein; Francisco-Ortega et al. 1996; Givnish 1998). Gene flow will be reduced among populations on geographically isolated islands compared to contiguous populations. When gene flow is absent or rare, populations may differentiate over time as a result of selection or genetic drift (Wright 1943; Mayr 1954). Thus, island systems are ideal for studies of both the processes that hold populations together (i.e., gene flow) and those that result in differentiation (i.e., selection, genetic drift).

In the Basin and Range province of the southwestern United States and northwestern México, a series of mountain ranges provide island-like habitats for plants and animals that require more mesic and temperate conditions than those offered by the deserts, scrublands and grasslands that now occupy the intervening basins (Marshall 1957). These Madrean sky islands developed as a series of deformation mountains produced by continental rifting that occurred between 12 and 6 million years BP (Nations and Stump 1981; Morrison 1991). At present, 17 Madrean sky islands are high enough to support mixed conifer forest (ca. 2500 m elev.,

somewhat lower toward the north and higher toward the south). They form an archipelago that connects extensive and continuous highlands to the north (the Central Mountain Province and the Colorado Plateau above the Mogollon Rim in Arizona) and south (the Sierra Madre Occidental that extends to within ~75 km of the Arizona-México border) (Fig. 1). As first documented by Shreve (1915; see also Heald 1950; Niering and Lowe 1984; Brown 1994; Warshall 1995), the Madrean sky islands support almost the same range of vegetation zones as occurs in all of the western United States, from open juniper woodlands just above semi-desert grasslands to spruce-fir forest on the summits of the highest mountains.

Here we report morphological differentiation among populations of *Castilleja austromontana* Standl. & Blumer (Scrophulariaceae), an herbaceous perennial that occurs on most of the Madrean sky islands. *Castilleja austromontana* is found above 2050 m elev., and therefore occurs as isolated populations in the highest of sky island habitats. We used data from vegetative, floral and seed characters for individuals from six sky island populations to address three questions: (1) Are populations distinguishable by the examined morphological traits? (2) If so, which traits most strongly differentiate the populations? (3) Can morphological divergence among populations be related to geography?

MATERIALS AND METHODS

*Castilleja austromontana* is found as far north as the White Mountains of Arizona (Apache County) and the Sangre de Cristo Mountains of northern

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TABLE 1. LOCATION OF POPULATIONS OF *CASTILLEJA AUSTROMONTANA* FROM WHICH SAMPLES WERE TAKEN. Abbreviations following the name of each mountain range are those used in Tables 2 and 4.

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<i>Catalina Mountains</i> (Cat): Mount Lemmon, ca. 2 miles above Ski Valley, 2750 m elev., Pima County, Arizona. <i>S. Slentz 44</i> (ARIZ).
<i>Santa Rita Mountains</i> (SR): About 200 meters below Baldy Saddle, Old Baldy Trail, Mount Wrightson, 2500 m elev., Santa Cruz County, Arizona. <i>S. Slentz and L. McDade 40</i> (ARIZ).
<i>Pinaleno Mountains</i> (Pin): Hospital Flat, 2750 m elev., Graham County, Arizona. <i>S. McLaughlin and J. Bowers 6431</i> (ARIZ).
<i>Rincon Mountains</i> (Rin): Italian Ranch Trail, Italian Spring, Saguaro National Park East, 2400 m elev., Pima County, Arizona. <i>S. Slentz 48</i> (ARIZ).
<i>Huachuca Mountains</i> (Hua): Carr Canyon Trail, 0.75 miles above Reef Area Campground, 2400 m elev., Cochise County, Arizona. <i>S. Slentz 43</i> (ARIZ).
<i>White Mountains</i> (Wht): Lee Valley Recreation Area, ca. 3 miles south of Sunrise Lake on State Rt. 273, 2800 m elev., Apache County, Arizona. <i>S. Slentz 45</i> (ARIZ).

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New Mexico and at least as far south as the limit of the sky island region in the northern Mexican states of Sonora and Chihuahua. Nothing is known about the age of the species nor is there a phylogenetic hypothesis for *Castilleja*. Flowering of *Castilleja austromontana* coincides with the summer wet season (July–September), and plants die back to the ground with the onset of winter. Our observations (44 hours on the Huachuca, Catalina and White Mountains) indicate that flowers of *C. austromontana* are visited and likely pollinated by hummingbirds. Individuals of *Selasphorus rufus* (Rufous Hummingbird) and possibly *S. platycercus* (Broadtailed Hummingbird) were observed to contact anthers and stigmas, and patches of pollen were seen on the heads of some visiting birds. There have been no detailed studies of the breeding systems of plants of this species. The seeds of *C. austromontana* have no special dispersal mechanism and seem to fall passively from dehiscing fruits. All *Castilleja* studied to date are autotrophic root parasites (Heckard 1962, Marvier 1996); nothing is known about the nutritional status or hosts of *C. austromontana*.

*Collection Sites and Protocol.* Field sites were located from specimens at the University of Arizona herbarium (ARIZ). We collected plants from one area on each of six different mountain ranges (Table 1; Fig. 1). During 1995 and 1996, we made at least one collecting trip to each mountain range during the flowering season to gather specimens for scoring vegetative and floral characters. These were followed by a later trip to collect mature fruits. Individual flowering plants were sampled by first determining the extent of the population, then walking a transect along the longest dimension of the population and collecting from individuals at intervals chosen to achieve the desired sample size (15–20 individuals per population) across this longest dimension. Individual plants produce one to many stems from a root crown and are clearly distinct from other individuals. One flowering stem from each individual was snapped off just above the root stock at ground level. Only fresh flowers and their subtending bracts were scored (freshness was as-

essed by presence of pollen on recently dehisced anthers).

Plants sampled for seed collection were chosen in the same manner. Infructescences from six to 11 individuals were collected from each population except the White Mountains, where mature fruits could not be collected. Seeds were sampled only from mature, partially dehisced capsules; only filled, apparently viable seeds were used. Because individuals from which flowering stems were collected were not marked, it was not possible to collect infructescences from the same individuals.

*Morphometrics.* A total of 41 characters was recorded from each flowering stem (35 measurements + 6 meristics); of these, 11 were vegetative and 30 were floral characters (Fig. 2, Appendix 1). Data were recorded from four leaves, three bracts, and three flowers per individual (Fig. 2). The sampling scheme for leaves and bracts specifically sought to capture positional differences in these structures: leaf and bract size and shape may vary along the stem or rachis, respectively, in *Castilleja*. We therefore included the measurements for each individual leaf and bract in the analyses. We calculated means of corolla and calyx measurements for the three flowers sampled from each plant and used these means in all analyses. In addition, to examine leaf and bract shape, we combined length and width measurements of individual leaves and bracts. The ratio of corolla to galea length varies in *Castilleja*; we determined this ratio for individual flowers and then calculated a mean value for each individual. The data set for univariate analysis thus includes a total of 39 characters (Table 2); of these, eight are ratios and the six calyx and corolla characters each represent means of three measurements. All measurements were log-transformed. Because entire bracts have cleft depths of zero, we added 0.5 to each value for depth of bract clefts prior to log-transformation. Meristic variables were square-root transformed.

The intricate surface characteristics of the seeds, as viewed with the SEM, have proven useful in taxonomic work with *Castilleja* (Chuang and Heckard 1992; Lockwood 1992). Initial experiments

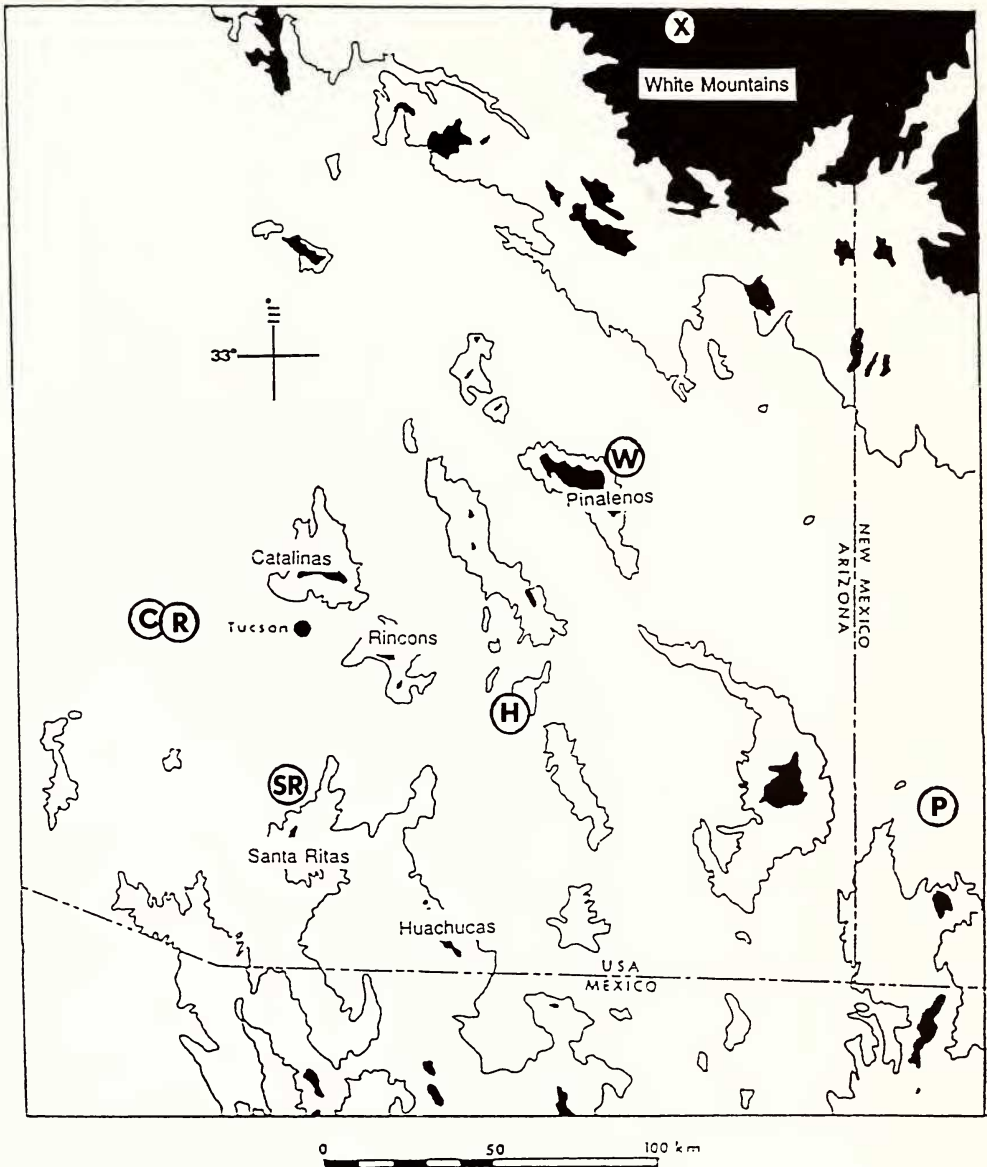


FIG. 1. The Madrean sky islands in southeastern Arizona and adjacent western New Mexico and northern Mexico. 1500 m contour lines outline mountain ranges. Shaded areas are >2500 m elevation, corresponding to approximate range of habitat of *Castilleja austromontana*. Circles indicate relative placement of plant populations by multidimensional scaling (the MDS result was scaled so that its dimensions matched those of the physical map; C = Catalina Mountains, H = Huachuca Mountains, P = Pinaleno Mountains; R = Rincon Mountains; SR = Santa Rita Mountains; W = White Mountains). An X indicates the collection location for the White Mountain population.

with techniques for preparing seeds (including dehydration and critical point drying) indicated that there were no differences between seeds prepared using these methods versus simply placed directly onto SEM specimen stubs. Several seeds from the same fruit were then placed onto a stub; stubs were coated with 30–60 nm of gold-palladium and viewed using an I.S.I. DS-130 scanning electron microscope. Several photographs were taken of at least one seed from each of 6–11 plants per popu-

lation for examination of seed characters. To facilitate standardization of measurements, seeds chosen to be photographed were in the same orientation, i.e., with the longest dimension of the seed parallel to the long axis of the photo. The SEM was periodically recalibrated by photographing a micrometer and adjusting the scale that appears on each photograph accordingly. Four measurements plus one meristic character were scored for each seed (Fig. 3, Appendix 1). To examine seed shape, we

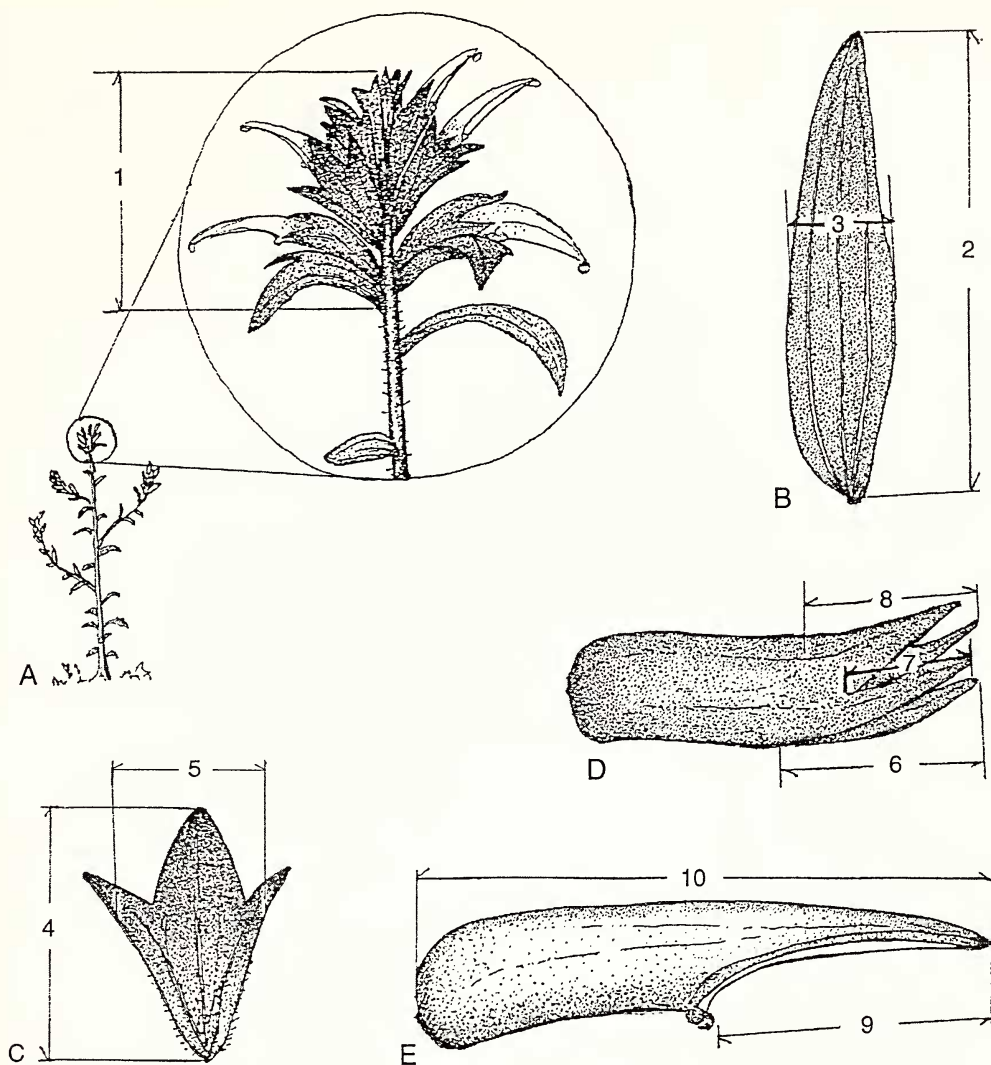


FIG. 2. *Castilleja austromontana*. A. Schematic illustration of plant with enlarged terminal raceme; B. leaf; C. floral bract; D. calyx; E. corolla. Numbered dimensions indicate measurements taken (Appendix 1, Table 1): 1 Raceme length; 2 Leaf length; 3 Leaf width; 4 Bract length; 5 Bract width; 6 Anterior calyx cleft depth; 7 Lateral calyx cleft depth; 8 Posterior calyx cleft depth; 9 Galea length; 10 Corolla length. Drawn by S. Slentz.

combined length and width measurements of individual seeds. When more than one seed was measured from an individual plant, within-plant means of each character were used in the analyses. As with the vegetative + floral data, measurements were log-transformed and meristic data were square-root transformed.

**Statistical Analyses.** The vegetative + floral data set was treated separately from the seed data because the plants from which seeds were taken were not matched with those from which flowering stems were taken. All analyses were conducted in SAS (SAS Institute 1988) except that NTSYS (Rohlf 1993) was used for multidimensional scaling (see below). One-way analyses of variance were used to determine which variables showed significant vari-

ation among populations. Student-Newman-Keuls a posteriori tests were used to assess which populations were significantly different from one another for each character. The vegetative + floral data set was subjected to principal components analysis (PCA) to examine relationships among characters. Ratios were omitted from the PCA because covariation between characters will be detected by multivariate methods automatically. Because only five seed variables were measured, the seed data were not analyzed using PCA. Correlation matrices were used for PCA because both metric and meristic characters were included.

Discriminant functions analysis (DFA) was used to determine how effectively the character data distinguish plants from different mountain ranges.

TABLE 2. RESULTS OF ANOVA FOR MORPHOLOGICAL CHARACTERS ORDERED BY PLANT STRUCTURE. Results of the Student-Newman-Keuls a posteriori test are reported for each variable, with populations ordered from high values to low. See Table 1 for key to abbreviations of mountains. Lines below mountain abbreviations connect populations that are not significantly different. Asterisks indicate p-values that are judged significant by the sequential Bonferroni test.

Character	Results of ANOVA	a posteriori test
<i>Vegetative:</i>		
Leaf length 1	F = 1.83; 5, 83 df; p = 0.1166	
Leaf length 2	F = 2.71; 5, 83 df; p = 0.0255	<u>Wht Pin Hua Cat SR Rin</u>
Leaf length 3	F = 3.64; 5, 83 df; p = 0.0050	<u>Wht Rin Pin Hua SR Cat</u>
Leaf length 4	F = 1.95; 5, 82 df; p = 0.0950	
Leaf width 1	F = 8.51; 5, 83 df; p < 0.0001*	<u>Pin Hua Rin SR Cat Wht</u>
Leaf width 2	F = 6.04; 5, 83 df; p < 0.0001*	<u>Pin Hua Cat SR Rin Wht</u>
Leaf width 3	F = 2.01; 5, 83 df; p = 0.0858	
Leaf width 4	F = 1.92; 5, 82 df; p = 0.0999	
Leaf length/width 1	F = 0.48; 5, 83 df; p = 0.7901	
Leaf length/width 2	F = 5.45; 5, 83 df; p = 0.0002*	<u>Wht Cat Hua SR Rin Pin</u>
Leaf length/width 3	F = 6.12; 5, 83 df; p < 0.0001*	<u>Wht Rin Hua Cat SR Pin</u>
Leaf length/width 4	F = 3.78; 5, 82 df; p = 0.0039	<u>Wht Rin Hua Cat Pin SR</u>
No. lateral branches	F = 4.84; 5, 49 df; p = 0.0011*	<u>Cat Rin Hua SR Pin Wht</u>
Apical stem width	F = 4.51; 5, 77 df; p = 0.0012*	<u>Rin SR Hua Pin Wht Cat</u>
Basal stem width	F = 3.12; 5, 78 df; p = 0.0129	<u>Hua Cat Rin Pin Wht SR</u>
<i>Reproductive:</i>		
No. of racemes	F = 3.64; 5, 63 df; p = 0.0059	<u>Rin Cat Hua Pin SR Wht</u>
No. of flowers	F = 1.95; 5, 82 df; p = 0.0943	
Raceme length	F = 1.65; 5, 82 df; p = 0.1572	
Bracts:		
Bract length 1	F = 2.34; 5, 83 df; p = 0.0489	<u>Wht SR Rin Hua Pin Cat</u>
Bract length 2	F = 2.74; 5, 83 df; p = 0.0244	<u>SR Wht Pin Rin Hua Cat</u>
Bract length 3	F = 2.95; 5, 83 df; p = 0.0168	<u>Wht SR Pin Rin Hua Cat</u>
Bract width 1	F = 1.20; 5, 83 df; p = 0.3185	
Bract width 2	F = 1.62; 5, 83 df; p = 0.1646	
Bract width 3	F = 2.56; 5, 83 df; p = 0.0332	<u>SR Wht Hua Rin Cat Pin</u>
Length/Width 1	F = 2.57; 5, 83 df; p = 0.0325	<u>Wht Rin Hua Pin SR Cat</u>
Length/Width 2	F = 2.09; 5, 83 df; p = 0.0753	
Length/Width 3	F = 3.88; 5, 83 df; p = 0.0033	<u>Pin Wht Rin Cat Hua SR</u>
Mean cleft depth 1	F = 4.49; 5, 83 df; p = 0.0011*	<u>Pin Cat Wht Hua SR Rin</u>
Mean cleft depth 2	F = 4.31; 5, 83 df; p = 0.0016	<u>Wht Pin Hua Cat SR Rin</u>
Mean cleft depth 3	F = 4.49; 5, 83 df; p = 0.0011*	<u>Wht Pin Hua Cat SR Rin</u>
No. of clefts 1	F = 3.41; 5, 83 df; p = 0.0075	<u>Pin Cat Wht SR Hua Rin</u>
No. of clefts 2	F = 2.26; 5, 83 df; p = 0.0560	
No. of clefts 3	F = 3.36; 5, 83 df; p = 0.0082	<u>Wht Pin SR Cat Hua Rin</u>
Calyx:		
Mean anterior cleft	F = 7.11; 5, 83 df; p = 0.0001*	<u>Wht Pin Hua SR Rin Cat</u>
Mean lateral cleft	F = 6.29; 5, 83 df; p = 0.0001*	<u>Pin Wht SR Hua Cat Rin</u>
Mean posterior cleft	F = 9.80; 5, 83 df; p = 0.0001*	<u>Wht Pin SR Hua Rin Cat</u>
Corolla:		
Mean corolla length	F = 8.62; 5, 83 df; p = 0.0001*	<u>Pin Hua Wht SR Rin Cat</u>
Mean galea length	F = 3.21; 5, 83 df; p = 0.0106	<u>Pin Wht Hua Rin SR Cat</u>
Mean corolla/galea	F = 3.56; 5, 83 df; p = 0.0057	<u>SR Pin Hua Wht Cat Rin</u>
<i>Seeds:</i>		
Seed length	F = 18.75; 4, 37 df; p < 0.0001*	<u>Pin Hua Cat Rin SR</u>
Seed width	F = 4.52; 4, 37 df; p = 0.0022	<u>Pin Cat Hua Rin SR</u>
Seed length/width	F = 1.10; 4, 37 df; p = 0.3724	
No. midline cells	F = 29.09; 4, 37 df; p < 0.0001*	<u>Pin Cat SR Hua Rin</u>
No. layers of testa	F = 15.28; 4, 37 df; p < 0.0001*	<u>Cat Hua Pin SR Rin</u>
Wall width	F = 12.13; 4, 37 df; p < 0.0001*	<u>Pin Hua Rin Cat SR</u>

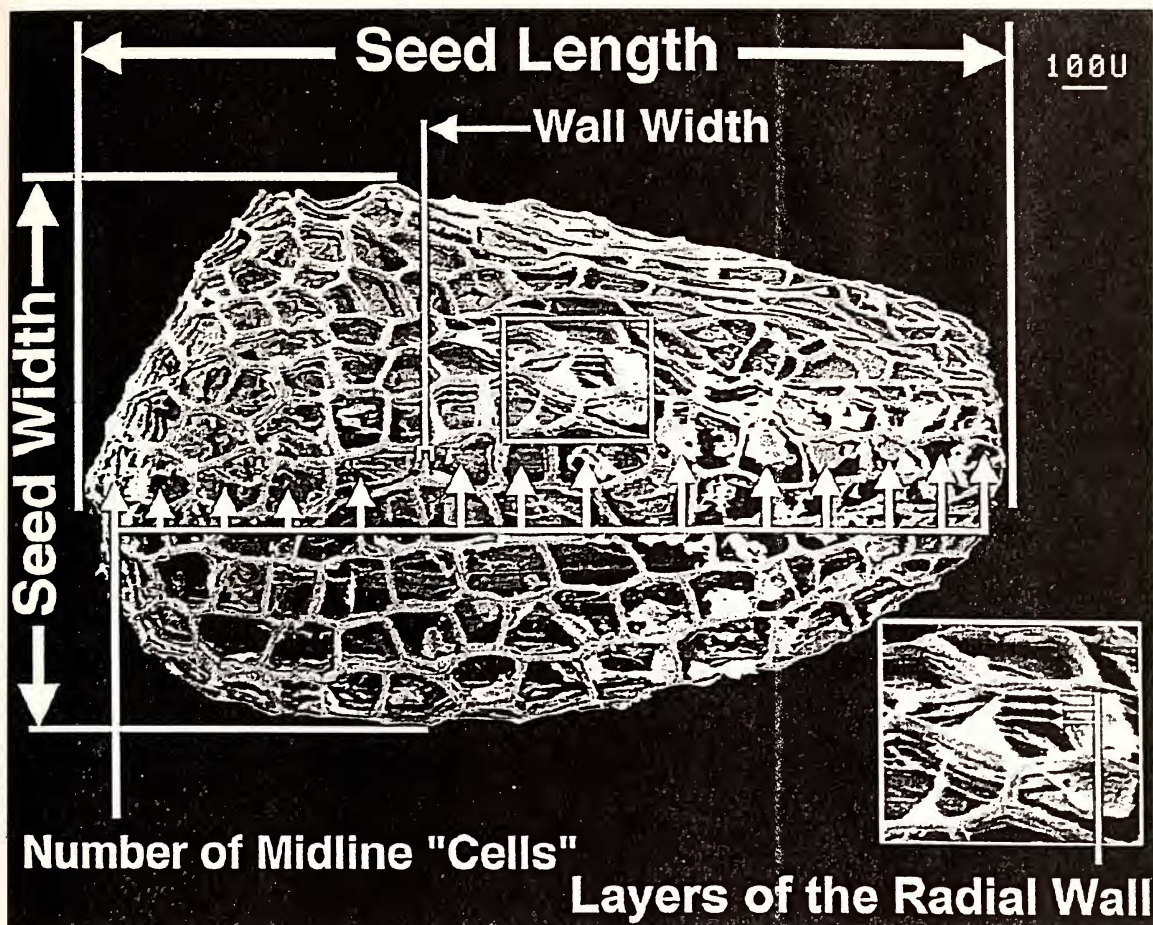


FIG. 3. Scanning electron micrograph of seed of *Castilleja austromontana*; seed length, width, and wall width were measured as indicated. Wall width was measured on a cell perpendicular to the line of vision. Number of midline cells = 14 on this seed as indicated by arrows. Enlarged cell in box illustrates layers of the radial walls (three in this case).

Character data were log transformed only if their distributions were non-normal as DFA is not affected by scaling of individual variables (Manly 1994). For seven plants from the Pinaleños, basal and apical stem widths were not measured; as a result, these plants were excluded by DFA. To maximize the number of plants included, we conducted an additional DFA excluding the two characters that were missing for these plants.

Non-metric multidimensional scaling (MDS) creates map-like diagrams that optimize the placement of each population relative to all others; the algorithm minimizes "stress," measured as the difference between pairwise distances in the diagram produced by MDS and the original distances. MDS is widely used in the social and biological sciences; a number of authors have used this technique to study the relationship between genetic or phenotypic distance and geographic distance (e.g., Wright and Ladiges 1997; Krauss 1996; Ruiz-Garcia 1997). Guiller, Bellido, and Madec (1998) found MDS to be superior to other ordination methods;

these authors provide a useful overview of the method and an explanation of its performance. Here we use MDS as a heuristic technique to visualize patterns of phenotypic distance in the context of geographic distances. Pairwise Mahalanobis distances between populations were calculated in SAS and entered into NTSYS for the MDS analysis for each of the two *Castilleja* data sets. We scaled the diagram produced by this analysis so that its dimensions matched those of the physical map depicted in Fig. 1. The MDS results were then overlaid on the geographic map of the sky island region to compare relative phenotypic and geographic distances (Fig. 1).

## RESULTS

Twenty-eight of 39 vegetative + floral characters and all seed characters except ratio of seed length/width varied significantly among populations (Table 2). Twelve vegetative + floral characters and four seed characters vary significantly among pop-

TABLE 3. EIGENVECTORS ON FIRST THREE PRINCIPAL COMPONENTS FOR CHARACTERS INCLUDED IN PRINCIPAL COMPONENT ANALYSIS OF THE VEGETATIVE AND REPRODUCTIVE DATA EXCLUDING SEEDS AND RATIOS. Analysis included all characters. The first three principal components account for 52.9% of the total variation. See Appendix 1 for explanation of characters.

Characters	PC1	PC2	PC3
Bract length 2	0.2736	0.1076	-0.1530
Leaf length 4	0.2703	0.0622	-0.0533
Mean anterior calyx cleft depth	0.2653	-0.0975	-0.0242
Leaf length 3	0.2630	0.0495	-0.0786
Bract length 1	0.2619	0.1452	-0.1370
Mean posterior calyx cleft depth	0.2528	-0.1973	0.0011
Mean corolla length	0.2461	0.0168	-0.0076
Bract length 3	0.2402	-0.0080	-0.2036
Bract width 2	0.2344	-0.0382	0.1694
Leaf width 3	0.2262	0.0857	0.1295
Leaf width 4	0.2188	0.1475	0.1563
Mean galea length	0.1916	0.0443	-0.0159
Raceme length	0.1907	0.0358	0.1193
Number of racemes	-0.1825	0.1042	0.2325
Number of lateral branches	-0.1807	0.1228	0.2234
Bract width 1	0.1739	0.2196	0.1607
Bract width 3	0.1731	0.1393	0.0733
Mean lateral calyx cleft depth	0.1609	-0.1291	-0.0052
Leaf length 2	0.1422	0.0328	0.0363
Number of flowers per raceme	0.1244	-0.0002	0.2762
Bract 3, mean cleft depth	0.1225	-0.2549	0.1069
Bract 2, mean cleft depth	0.1204	-0.3384	0.1773
Leaf width 2	0.1194	0.2331	0.1976
Bract 2, number of clefts	0.1027	-0.2743	0.2194
Bract 3, number of clefts	0.0592	-0.3596	0.2684
Leaf length 1	-0.0502	0.2115	0.2808
Basal stem width	-0.0415	0.1710	0.3017
Leaf width 1	-0.0305	0.3290	0.2622
Bract 1, number of clefts	-0.0295	-0.2443	0.2698
Bract 1, mean cleft depth	0.0266	-0.2354	0.2871
Apical stem width	-0.0262	0.1100	0.1219
Eigenvalue	8.95	4.14	3.32
% variance explained	28.88%	13.36%	10.69%

ulations even after adjusting threshold probability values via the sequential Bonferroni test (Rice 1989) (Table 2). Characters that vary significantly among populations are from diverse plant structures (Table 2). Leaf shape (i.e., length/width) and size differ among populations, with plants from the White Mountains having leaves that are relatively longer and narrower than those of plants from the other sky islands. Plants from the White Mountains also have relatively few branches. Bracts differ in cleftedness (i.e., both number and depth of clefts), as well as in shape (length/width) and length among sky islands. Plants from the White Mountains and the Pinaleños have calyces that are more deeply cleft than those of other sky island populations. Corollas differ in total length and in galea length; flowers of plants from the Pinaleños are largest whereas those from the Catalinas are smallest. Plants from the Pinaleños have long, wide seeds with many cells along the midline and wide walls compared to seeds from plants from the Santa Ritas or Rincons. Seeds from plants from the Catalinas have the most layers of the radial wall and those

from the Rincons the fewest; seeds from plants in the Pinaleños are intermediate for this character.

For the analysis including vegetative + floral data, the first three principal components each account for >10% of the variation among individuals, but together explain only 52.9% of total variation. PC1 accounts for 28.9% of the standardized variance in the data. The characters that contribute most to PC1 are length of bract 2, depths of the calyx clefts, and corolla length (Table 3). Raceme length, bract length and width, and leaf length and width also contribute substantially. The characters contributing most to PC2 involve cleftedness of bracts (i.e., both number and depth of clefts), and bract length (Table 3). This component explains an additional 13.4% of the standardized variance. Characters contributing most to PC3 are basal and apical stem width, number of flowers per raceme, and leaf width. The third principal component accounts for 10.7% of the standardized variance.

The next two principal components each account for more than 5% of the total variation for a cumulative total of 68% of total variation accounted

TABLE 4. NUMBER (ABOVE) AND PROPORTION (BELOW) OF PLANTS FROM SOURCE LOCATIONS (LEFT) THAT WERE IDENTIFIED BY DISCRIMINANT FUNCTIONS ANALYSIS OF VEGETATIVE + FLORAL DATA AS FROM THE LOCATIONS LISTED ABOVE. Characters were excluded for which data were missing for some plants. See Table 1 for key to abbreviations of mountains.

	Cat	Hua	Pin	Rin	SR	Wht
Cat	10 <b>76.9</b>	1 7.7	0 0.0	2 15.4	0 0.0	0 0.0
Hua	1 7.7	12 <b>92.3</b>	0 0.0	0 0.0	0 0.0	0 0.0
Pin	0 0.0	0 0.0	15 <b>100.0</b>	0 0.0	0 0.0	0 0.0
Rin	1 9.1	0 0.0	0 0.0	8 <b>72.7</b>	2 18.2	0 0.0
SR	1 5.6	0 0.0	0 0.0	1 5.6	16 <b>88.9</b>	0 0.0
Wht	0 0.0	0 0.0	1 6.3	0 0.0	0 0.0	15 <b>93.8</b>

for by the first five principal components. Sixteen principal components must be considered in order to explain more than 95% of the variation. Following Legendre and Legendre (1998) and considering only PCs with eigenvalues >1 restricts us to the first eight PCs which account for only 81% of the variation. Although there are no established guidelines for judging the power of a PCA to explain variation, Sneath and Sokal (1973) suggest that three PCs satisfactorily illustrate patterns of variation in most systematic studies. This is not the case here, indicating that many characters vary independently of others such that morphological variation among sky island populations of *Castilleja* defies reduction to a few orthogonal principal components.

Discriminant functions analysis including all plants (i.e., omitting basal and apical stem width which were missing for seven plants from the Pinaleños) indicated that plants can be linked to their source population with considerable accuracy: 88% (76 of 86 plants) are correctly assigned (Table 4). Nine of ten errors involved plants from the southern four populations (i.e., the Catalinas, Rincons, Santa Ritas and Huachucas) and no plants from the Pinaleños were misplaced.

Seeds of *C. austromontana* are of Lockwood's (1992) Type 1; the outer membrane is essentially completely ruptured and lost at maturity, the outer tangential wall is smooth, and the radial walls of the "cells" are unornamented. Seed characters vary among populations (Table 2) and discriminant functions placed more than ¾ of plants correctly based on seed characters (Table 5). Errors were most frequent regarding seeds from the Huachucas; nearly half were incorrectly placed. However, no seeds from the Pinaleños were misclassified as being from other sky islands nor were seeds from other populations placed on the Pinaleños. Seed mor-

TABLE 5. NUMBER (ABOVE) AND PROPORTION (BELOW) OF PLANTS FROM SOURCE LOCATIONS (LEFT) THAT WERE IDENTIFIED BY DISCRIMINANT FUNCTIONS ANALYSIS OF SEED DATA AS FROM THE LOCATIONS LISTED ABOVE. See Table 1 for key to abbreviations of mountains.

	CAT	HUA	PIN	RIN	SR
CAT	5 <b>62.5</b>	1 12.5	0 0.0	1 12.5	1 12.5
HUA	1 9.1	6 <b>54.6</b>	0 0.0	2 18.2	2 18.2
PIN	0 0.0	0 0.0	9 <b>100.0</b>	0 0.0	0 0.0
RIN	0 0.0	0 0.0	0 0.0	6 <b>100.0</b>	0 0.0
SR	2 25.0	0 0.0	0 0.0	0 0.0	6 <b>75.0</b>

phology among plants on the Pinaleños is distinctive; these seeds are large with wider walls than those of plants from other sky islands.

Superimposing the diagram from the MDS analysis of vegetative + floral characters on the map of the sky island region indicates that, among the Catalinas, Rincons, Santa Ritas, and Huachucas, physical and relative phenotypic distances are roughly congruent (Fig. 1). Relative to these four, the White Mountains are placed nearer and Pinaleños farther by MDS based on phenotypic distances than their geographic distances suggest.

The MDS analysis of seed data (not shown) placed the Pinaleño population distantly from the other four populations which were proximate and approximately equidistant from each other.

## DISCUSSION

Our results demonstrate morphological differentiation among populations of *Castilleja austromontana* on Madrean sky islands. DFA associated 88% of plants with the population from which they were collected, and PCA indicates that many vegetative and reproductive characters contribute to this differentiation. At one extreme, plants on the White and Pinaleño Mountains tend to be relatively small in stature, with long leaves and bracts, long corollas, and deep bract and calyx clefts. At the other extreme, on the Catalina and Rincon Mountains, plants tend to be relatively tall with smaller leaves and flowers, and shallow clefts in bracts and calyces. Plants on the Huachuca and Santa Rita Mountains have structures that are intermediate in size and shape between these extremes.

It is important to note that we do not know the cause of this morphological variation. It is possible that the characters are phenotypically plastic and that our results reflect environmental differences among mountains. These plants are likely root parasites, so that common garden or reciprocal sowing or transplant approaches to examine this possibility will be difficult at best. Because the sizes and shapes of reproductive structures are generally un-



derstood to be under selection associated with achieving successful pollination and thus reproduction, these are often less phenotypically plastic than vegetative characters (e.g., Bradshaw 1965; Davis 1983; Apelgren and Lernstal 1991). Our results indicate that both vegetative and floral characters vary among sky island populations, suggesting that we are not dealing solely with phenotypic variants. However, data from other sources will be necessary to confirm that the differentiation that we see is genetically based. To the extent that the characters studied here have a genetic basis, they may also be genetically correlated and thus not reflect a suite of independent genetic differences. However, PCA suggests that many characters are independent of each other, at least in their phenotypic expression.

Selection, gene flow, and genetic drift may all contribute to the patterns of differentiation that we have documented. Contrasting selective regimes may be responsible for the morphological differences among plants from different sky island populations. We have no basis at present for specific hypotheses regarding the direction or magnitude of selection except that disruptive selection on corolla morphology is unlikely because pollinator relationships appear to be uniform.

Some inferences regarding gene flow are possible based upon geographic distances, pollinator relationships, and vegetation history. If gene flow (whether by pollen or migrants) is negatively related to geographic distance, then proximate populations should be more similar than populations that are distant and thus rarely exchange genes. The physical distances among the Catalina, Rincon, Santa Rita, and Huachuca populations are fairly congruent with phenotypic distances among plant populations from these ranges (Fig. 1) in accord with a gene flow or isolation by distance explanation. In contrast, the placement by MDS based on phenotypic distance of the White Mountain and Pinaleno populations relative to the other four is not congruent with geographic distances (Fig. 1). In this light, the behavior of pollinators may be informative.

As described above, observations suggest that the same hummingbird species visit flowers of *C. austromontana* across the species' range. Individuals of *Selasphorus platycercus* breed on the sky islands at the elevation of the *Castilleja* populations and thus should move pollen within ranges. In contrast, Rufous-tailed Hummingbirds (*S. rufus*) pass through these mountains during the late summer and early fall migration south to their winter range (the birds fly at lower elevation during their spring migration north because they precede the spring thaw at high elevation) (Grant and Grant 1967; Johnsgard 1983). If relative phenotypic distance reflects gene flow, then our data suggest a more direct route between the southern populations and the White Mountains than would be expected based on geographic distance. Unfortunately, migration

routes are understood only in broad geographic terms, not at the level of routes followed by individual birds.

Vegetation history of these mountain ranges yields some ideas about past patterns of gene flow. During the late Pleistocene and Holocene, cycles of climate change were associated with glaciations farther north and at higher elevations. Fossil packrat middens provide direct evidence of vegetation changes associated with climatic changes during the last 40,000+ years (Van Devender, Thompson and Betancourt 1989; Betancourt et al. 1990 and references therein). During periods of glacial maxima, vegetation zones on the sky islands were substantially lower than they are today. The most complete evidence, from the most recent glacial maximum (i.e., 20,000 to 8,000 years BP), indicates that the habitat in which *C. austromontana* occurs ranged 500–600 m lower than at present. This would presumably have increased the area of suitable habitats on the sky islands and also increased connectivity among them. Higher connectivity would have likely increased gene flow between populations that are proximate (e.g., Catalinas and Rincons) and between those that are separated by valleys of relatively high elevation (e.g., Huachucas and Santa Ritas). Both the geographic distance and topographic complexity between these four sky islands and the Pinalenos and White Mountains preclude simple predictions of the impact of climate change on gene flow.

Finally, genetic drift occurs in populations that are isolated, especially if they are small (Lande 1976; Hartl and Clark 1989). We do not have census data for these populations, but some inferences are possible based upon areal extent of suitable habitat. The area inhabited by *C. austromontana* in the White Mountains is vast such that this population should be least susceptible to chance effects. In contrast, the area of suitable habitat for *C. austromontana* on all of the sky islands is far more limited and all of these populations may be subject to drift. This is especially true if warm interglacial periods produced an upward elevational shift in climatic zones comparable to the well-documented downward shift associated with glacial maxima.

Lande (1976:32) derived an equation to estimate the population size ( $N^*$ ) below which drift may influence morphological evolution:

$$N^* = \frac{(1.96)h^2t}{(z/\delta)^2}$$

where  $h^2$  is heritability,  $t$  is number of elapsed generations,  $z$  is the difference in character means between two samples (i.e., the magnitude of morphological change observed), and  $\delta$  is the standard deviation of the character. We estimated values for this population size threshold in *C. austromontana* using our data for corolla length from the Huachucas and Santa Ritas. Heritability has not been estimated for corolla length in *C. austromontana*, but

Campbell (1996) obtained  $h^2$  values between 0.24 and 0.74 for another Asteridae, *Ipomopsis aggregata* (Polemoniaceae). It seems likely that  $h^2$  for corolla length in *C. austromontana* falls within this rather large range. Further, we can estimate time since these populations were in contact: because of the relatively high basin between these two ranges, we hypothesize that there would have been nearly continuous habitat for *C. austromontana* during the last glacial maximum, ca. 10,000 years BP. Generation time is not known for these plants and we used 5 and 10 yr values as reasonable for herbaceous perennials. The range of parameters explored gives estimates from 21,000 ( $h^2 = 0.24$ , generation time = 10 yr) to 130,000 individuals ( $h^2 = 0.74$ , generation time = 5 yr); that is, drift cannot be ruled out if population sizes are lower than these values. Based on field experience and on areas of suitable habitat among sky islands, it seems likely that only the White Mountains could have as many as 20,000 individuals. These results are consistent with the hypothesis that drift is at least partly responsible for the differences that we see among sky island populations.

There are no other quantitative studies of plant population differentiation among Madrean sky island plants. Maddison and McMahon (in press) have documented substantial morphological and behavioral differences among male spiders (*Habronattus*: Salticidae) on different sky islands. Snails of the genus *Sonorella* (Helminthoglyptidae; Miller 1967) and beetles (*Scaphinotus*, Carabidae; Ball 1966) are reported to be highly differentiated among the Madrean sky islands although quantitative or phylogeographic studies are lacking. Such studies are underway for spiders (S. Masta personal communication) and lizards and snakes (K. Zamudio and H. Greene, Cornell University, personal communication). Other sky island systems (e.g., the tepuis of Venezuela) have been the focus of floristic and evolutionary studies above the species level (Givnish et al. 1997), but we are not aware of any research to document intraspecific patterns of differentiation there. Plant populations on true island systems have been studied by a number of researchers, but patterns of intraspecific differentiation have received relatively little attention (but see Francisco-Ortega et al. 1993; Hotta et al. 1985), in part because there appears to be little genetic divergence within many island lineages (Givnish 1998).

In sum, our results confirm that the Madrean sky islands are likely to be evolutionary hot spots and that further research is warranted. In the case of *C. austromontana*, additional populations should be studied, particularly from the southern portion of the Madrean sky island province (i.e., the Mexican sky islands between the international border and the "mainland" of the Sierra Madre Occidental). Genetic data that can be ordered (e.g., DNA sequences) would permit us to test more explicitly our ideas regarding gene flow and genetic drift, as well as to add a phylogeographic component (sensu Avise

1989, 1994; Templeton 1998). Further, parallel studies on other groups of plants with both similar and contrasting life histories, pollination systems and dispersal mechanisms will deepen our understanding of both the correlates and determinants of population differentiation.

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APPENDIX 1. EXPLANATION OF CHARACTERS MEASURED OR COUNTED FOR *CASTILLEJA AUSTROMONTANA*. See Figures 2 and 3 for illustrations of structures measured. Terminology follows Chuang and Heckard (1992).

*Vegetative:*

*Basal Stem Width* was measured just above the root stock.

*Apical Stem Width* was measured directly below the inflorescence.

*Leaf Length* (Fig. 2B-2) and *Leaf Width* (Fig. 2B-3) were measured for four leaves per sampled stem including the most proximal (leaf 1), most distal (i.e., last vegetative leaf below the most proximal floral bract; leaf 4), and two intermediates at ca.  $\frac{1}{3}$  and  $\frac{2}{3}$  of total stem height.

*Number of Lateral Branches* includes both vegetative and reproductive branches.

*Floral:*

*Number of Racemes* is number of branches bearing an inflorescence.

*Number of Flowers* on the raceme selected for measurement of floral characters.

*Raceme Length* was measured from directly below the most proximal floral bract to the tip of apical bud (Fig. 2A-1).

*Bract, Calyx and Corolla:* three nodes were sampled and scored for bract, calyx and corolla characters; these were the most proximal (1), most distal (3), and approximately median (2) flowering nodes.

*Bract Length* (Fig. 2C-4) and *Bract Width* (Fig. 2C-5)

*Number of Clefts per Bract* was counted on each of the three sampled bracts; bracts have from zero (entire) to four deep clefts on each lateral margin.

*Mean Cleft Depth per Bract* of all clefts on a given bract. Because number and position of clefts varied among bracts, it was not possible to compare individual clefts. This variable attempts to capture 'cleftedness,' which clearly varied among plants, without comparing individual clefts.

*Calyx:* Depths of the anterior (Fig. 2D-6), lateral (Fig. 2D-7), and posterior (Fig. 2D-8) calyx clefts were measured as the straight line distances from the base of each cleft to the apex of an adjacent calyx lobe. For analysis, measurements for the three sampled calyces were combined as mean depths of anterior, lateral and posterior clefts.

*Corolla Length* is from the apex of the posterior lobes of the corolla to the posterior base of the corolla (Fig. 2E-10). For analysis, measurements for the three sampled corollas were combined as mean corolla length.

*Galea Length* is from the apex of the posterior lobes of the corolla across the mouth of the corolla to the apex of the anterior lip (Fig. 2E-9). For analysis, measurements were combined as mean galea length.

*Seeds:* All variables were scored from SEM photomicrographs (Fig. 3). When more than one seed per individual was photographed, measurements or counts were averaged for analysis.

*Seed Length* was measured from the hilum to the opposite end of the long axis of the seed.

*Seed Width* was measured at the widest point of the seed as photographed in the standard orientation.

*Number of Layers of the Radial Wall* is the number of layers of "scalariform thickenings on the radial walls" (Lockwood, 1992:225).

*Number of Midline Cells:* We counted number of cells in the longest row along the midline of the seed.

*Radial Wall Width:* Width of the radial wall of testa cells measured at the widest point on a cell directly perpendicular to the line of sight.

*Derived Variables:* These ratios were calculated for each leaf, bract, and corolla measured; ratio of seed length/seed width was calculated based on within-plant mean length and width values (see Table 2).

*Leaf Length/Leaf Width:* This variable describes leaf shape; leaves with low ratios are more ovate than those with higher ratios.

*Bract Length/Bract Width:* This variable describes bract shape; bracts with low ratios are more ovate than those with higher ratios.

*Corolla length/Galea length:* This variable describes the corolla shape. Flowers with low ratios have long galeas relative to corolla length, and those with higher ratios have short galeas relative to corolla length.

*Seed Length/Seed Width:* This variable describes seed shape; seeds with low ratios are more ovate than those with higher ratios.